

October 25, 1954

Dear Dr. Pijper:

Thank you for your very kind letter of the 11th. I am happy to see that you did not misunderstand the purpose of my note on oil chambers, which was intended precisely for the purpose of acquainting and re-acquainting others with an old technique.

I am surprised that you have had trouble with this method. I have been delighted with the clarity of microscopic observation and with the rapid growth and regular viability of clones from single cells. In my own work, I have used almost exclusively *Escherichia coli*, *Salmonella* spp. and *Caulobacter* (for some incidental observations), and these should be presumably capable of growth under nearly anaerobic conditions. But Dr. Lwoff has had equally good luck with *Bacillus megaterium*. If necessary for strict aerobes one could inject a small bubble of air near the droplet with the bacteria, but I have never found this necessity. I have not yet done any ~~any~~ motion pictures, but I would have that that oil chamber preparations would be ideal for them. My own studies have been concerned especially with the apparent conjugation of *E. coli* cells that precedes genetic recombination, i.e., that is apparently the sexual process. The cells do not fuse, but separate after about an hour's association; the clones from erstwhile pairs show very high incidences of genetic recombination of the markers distinguishing the components.

I ~~do~~ do not believe that lack of oxygen is the primary trouble in your trials. The growth medium might be unsatisfactory in your situation (I have been using Difco "Penassay Broth", which is a moderately buffered yeast-extract-glucose concoction); possibly more likely, the mineral oil is toxic. This is especially likely if any attempt has been made to sterilize the oil, especially by heating. I have found this unnecessary, but you might prefer, after some control tests, to autoclave bottles of oil to which some distilled water has been added. It will then be necessary to let the bottled stand afterwards until the cloudy dispersion is dissipated.

~~I~~ I am sorry if my reference to a ~~Yeast/Peptone~~ "container of oil was puzzling", but I still do not see how. I had been using a small staining dish filled with oil as a receptacle for the cover-glasses carrying the droplers; as you would imagine (possibly here was the difficulty) this does not furnish good access to air, but has worked well nevertheless.

Under separate cover, I am sending this and other reprints, and will be delighted to continue the exchange. I shall also look forward to hearing from you of your success with this method when the probably minor obstacle is discovered. I am hoping to set up a motion picture apparatus to photograph the conjugations and will be receptive to any technical suggestions.

Yours sincerely,

Joshua Lederberg
Professor of Genetics